In nature most bacteria live as part of structured sessile communities growing on solid surfaces or interphases and embedded in a self-produced extracellular matrix, named biofilms. *Pseudomonas putida* is a rhizosphere-associated soil bacterium that forms biofilms on multiple biotic and abiotic surfaces. Biofilm formation by *P. putida* involves cessation of flagellar motility and the production of an extracellular matrix including the high molecular weight adhesins LapA and LapF and several types of extracellular polysaccharides. When nutrients become limitant, *P. putida* undergo quick dispersal, during which cells are released from the extracellular matrix and resume a motile lifestyle. Dispersal involves the proteolytic cleavage of LapA triggered by a drop in the intracellular concentration of the second messenger c-di-GMP. In this project we have identified three proteins, BifA, DksA and MvaB, as novel elements involved in the starvation-induced dispersal response. We have shown that the PDE BifA is responsible for decreasing c-di-GMP levels during nutrient starvation and triggering biofilm dispersal. BifA also contributes to the regulation of the steady-state c-di-GMP levels. Positive regulation of *bifA* expression by the stringent response mediators 5'(p)ppGpp and DksA and the flagellar σ factor FliA links nutrient limitation and the resumption of flagellar motility with c-di-GMP mediated dispersal. We have also studied the role of the c-di-GMP-responsive-s^{54}-dependent transcriptional factor FleQ as the switch between the planktonic and sessile lifestyles in *P. putida*. FleQ activates the expression of σ^{54}-dependent flagella and chemotaxis-related promoters and its activity is inhibited by c-di-GMP. At the same time, FleQ exerts a dual positive and negative regulation on the transcription of *lapA* and the cellulose biosynthesis operon in a s^{54}-independent manner, and in the case of *lapA* transcription is stimulated by c-di-GMP. In addition, FleQ and/or c-di-GMP also regulate the synthesis of several putative c-di-GMP-metabolizing enzymes likely contributing to the robustness of the regulatory circuit. Our results indicate that the switch between the planktonic and biofilm lifestyles involves the coordinate regulation of flagellar motility and synthesis and degradation of matrix components. Such regulation involves a plethora of regulatory and signal transduction elements, and is driven by changes in the intracellular levels of c-di-GMP.

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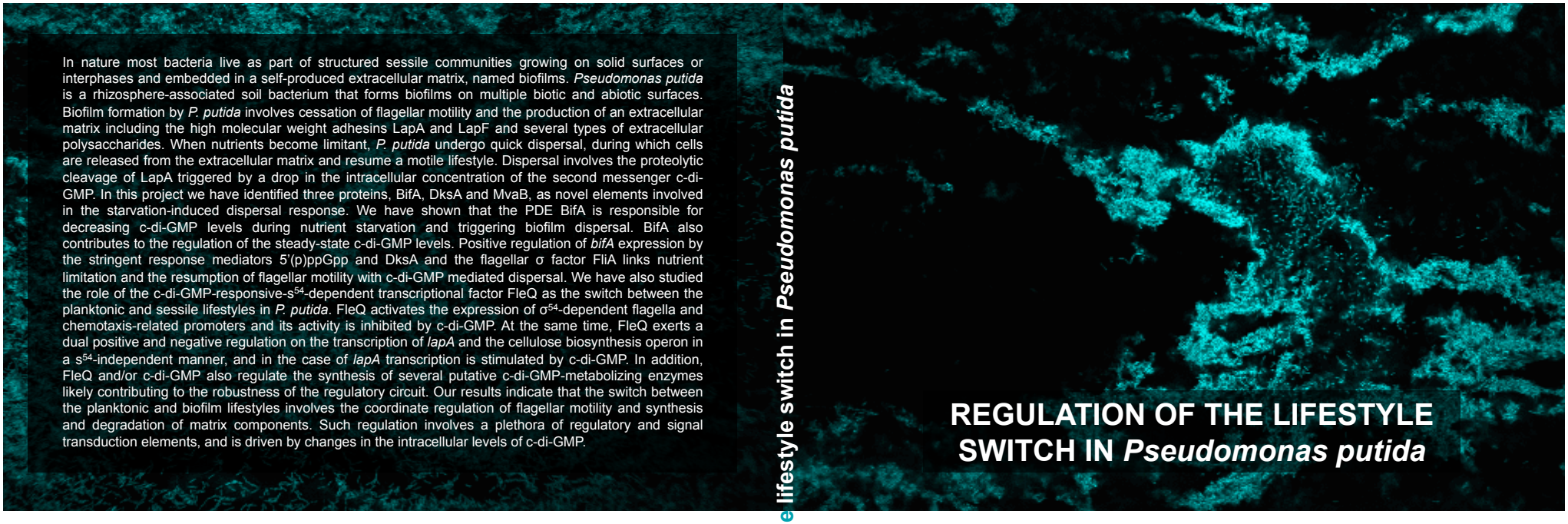
Regulation of the lifestyle switch in *Pseudomonas putida*

PhD THESIS | ALICIA JIMÉNEZ FERNÁNDEZ



REGULATION OF THE LIFESTYLE SWITCH IN *Pseudomonas putida*

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